Identification of the critical extracellular matrix proteins that promote human embryonic stem cell assembly.

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Authors: Denis Evseenko, Katja Schenke-Layland, Gautam Dravid, Yuhua Zhu, Qian-Lin Hao, Jessica Scholes, Xing Chao Wang, W Robb Maclellan, Gay M Crooks

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Public Summary:
Human embryonic stem cells (hESC) exist as large colonies containing tightly adherent, undifferentiated cells. Disaggregation of hESC colonies into dispersed single cells significantly reduces their survival and affects differentiation, suggesting that adhesion mechanisms are critical for the assembly and maintenance of hESC colonies. The goal of these studies was to determine which molecules control cell adhesion between single hESC. Our studies demonstrate that undifferentiated hESC produce two types of proteins laminin-511 and nidogen-1, both of which belong to a family of proteins called extracellular matrix proteins. When laminin and nidogen were added to single cell suspensions of hESC we were able to restore hESC assembly in the absence of mouse cells or exogenous chemicals. These data reveal for the first time the crucial role of the Extra cellular matrix proteins laminin-511 and nidogen-1 in hESC assembly, and provide a novel practical tool to investigate hESC differentiation in a natural microenvironment.

Scientific Abstract:
Human embryonic stem cells (hESC) exist as large colonies containing tightly adherent, undifferentiated cells. Disaggregation of hESC as single cells significantly affects their survival and differentiation, suggesting that adhesion mechanisms are critical for the assembly and maintenance of hESC colonies. The goal of these studies was to determine the key extracellular matrix (ECM) components that regulate assembly and growth of hESC. Our studies demonstrate that undifferentiated hESC express a specific subtype of laminin (laminin-511) and nidogen-1. The addition of a purified protein complex comprised of human laminin-511 and nidogen-1 to single-cell suspensions of hESC is sufficient to restore hESC assembly in the absence of murine embryonic fibroblasts or exogenous chemicals. The mechanism of hESC aggregation is through binding of the alpha6beta1 integrin receptor highly expressed in the membranes of undifferentiated hESC; aggregation can be inhibited by an antibody against alpha6 and almost completely blocked by an antibody against the beta1 subunit. Reassembly of defined numbers of purified hESC with the laminin-nidogen complex allows consistent production of uniform embryoid bodies (EBs) ("LN-EBs") that differentiate into endodermal, ectodermal, and mesodermal derivatives, and are highly efficient in generating hematoendothelial progenitors. These data reveal for the first time the crucial role of the ECM proteins laminin-511 and nidogen-1 in hESC assembly, and provide a novel practical tool to investigate hESC differentiation in a xenogen-free microenvironment.